

Modulation of Avian Responsiveness to Chemical Irritants: Effects of Prostaglandin E1 and Analgesics

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ABSTRACT Chemical irritation appears to be modulated by similar mechanisms in birds and mammals, despite an apparent difference between the two taxa for what constitutes a chemical irritant. Prostaglandin E1, a well-described mammalian pain modulating substance, was not itself aversive to starlings, although it did sensitize birds to the effects of the avian irritant o-aminacetophenone. Aspirin and aspirin-like drugs tended to desensitize starlings to the aversive effects of acetophenone bird repellents. Because the modulation mechanisms for the perception of pain appear to be similar in birds and mammals, the taxonomic differences in the perception of stimuli as irritating is inferred to be due to differences in receptor mechanism. The differences in sensory perception of chemical irritants has important implications for vertebrate foraging ecology and the evolution of plant-animal interactions. © 1995 Wiley-Liss, Inc.*

The perception of pain is presumed to be evolutionarily adaptive because it allows an animal to minimize its exposure to potentially harmful stimuli. Stimuli may be tactile, thermal, or chemical in nature and are mediated by nociceptors of the somatosensory and trigeminal systems (Erikson, '87). In the case of "slow pain," i.e., "burning pain," fibers are activated when tissue damage occurs, causing the release of endogenous substances such as peptides (e.g., bradykinin), amines (e.g., serotonin, histamine), and arachidonic acid derivatives (e.g., prostaglandins) (Terenius, '87). Each of these endogenous substances appears to be mediated by separate receptor mechanisms that generate the hyperalgesic response (Higashi et al., '82). Behaviorally, the hyperalgesic response is manifested as a withdrawal from the stimulus. Thus, one function of endogenous pain substances is to warn an organism about cell and tissue damage.

Exogenous chemicals, e.g., plant metabolites, insect defensive secretions, can cause pain for animals in several ways (Nielsen, '91). Chemo-irritants may act nonspecifically by causing physical damage to cells, thus setting forth a release of endogenous substances which specifically code for pain. Chemo-irritants also may invoke a pain response by nonspecifically activating chemoreceptors via electrostatic processes (e.g., induced proton flow across ion channels) initiated by physical proximity of the stimulus to the receptor. Thus, physicochemical properties of the irritant would govern access to the receptor through the mucosa

and epidermal layers, and electronic features of the molecule would activate the chemically sensitive nociceptor. Finally, chemical irritants may invoke a pain response by specifically binding to, and activating chemoreceptors located on the chemosensitive afferent. Whether there are chemoreceptors that have evolved as a consequence of selective pressures from exogenous chemicals or whether exogenous chemicals merely mimic properties of endogenous pain substances are important evolutionary considerations that have profound implications for understanding the evolution of plant-animal interactions.

The first step prior to considering this evolutionary question is to gain a better understanding of the comparative mechanistic relationships governing perception of pain among taxa. To this end, the effects of mammalian analgesics and sensitizing agents, i.e., prostaglandins, were studied to determine their effects on avian sensitivity to chemical irritants. Previous studies indicated that birds and mammals differ in their ability to perceive irritants (Mason et al., '91; Clark and Shah, '94; Mason and Clark, in press). Birds are insensitive to a variety of well-described mammalian irritants, e.g., capsaicin, ammonia, gingerol, zingerone, hydroquinones, and naphthalene (Szolcsanyi et al., '86; Dolbeer et al., '88; Mason and Otis, '90). For

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example, mammals uniformly avoid concentrations of 100–1,000 ppm of capsaicin (the hot component of red chilies: Mason et al., '91), yet birds are indifferent to concentrations up to 20,000 ppm (Szolcsanyi et al., '86). Other familiar mammalian irritants are also irritating to birds, but only at high concentrations (>10,000 ppm), and under specific presentation paradigms, e.g., piperine, allyl isothiocyanate, and mercaptobenzoic acid (Mason and Otis, '90). Sensitivity to only high concentrations suggests that these stimuli nonspecifically activate chemoreceptors. Similarly, potent avian chemical irritants, represented by a variety of acetophenones and anthranilates (Clark and Shah, '91, '94; Clark et al., '91), are generally not irritating to most mammals (Mason et al., '91). This study focuses on mechanisms for the modulation of irritant perception in birds.

MATERIALS AND METHODS

Animals

Starlings (*Sturnus vulgaris*) were chosen as test animals because previous experiments showed them to be good models of avian chemical sensitivity (Clark and Shah, '91). Birds were captured at Sandusky, Ohio, and transported to the Monell Center. Upon arrival, each was individually caged (61 × 36 × 41 cm) under a 12:12 light:dark cycle for at least a 2-week adaptation period and given free access to Purina Flight Bird Conditioner (Purina Mills, St. Louis, MO), water, and oyster shell grit (United Volunteer Aviaries, Nashville, TN). Capture, maintenance, and experimental protocol were carried out in compliance with guidelines set forth by the Institutional Animal Care Committee.

Pilot studies on prostaglandins

Various prostaglandins have been shown to sensitize mammalian and avian tissue to the effects of irritation (Ferreira and Vane, '74; Chromiak and Vandeburgh, '94). Furthermore, the role of prostaglandins in birds seems to parallel the function seen in mammals (e.g., Macari et al., '93; Kotwani et al., '94; Lundholm, '93). Based upon these reports and on evidence for synthesis of specific prostaglandins in birds (Kotwani et al., '93; Chromiak and Vandeburgh, '94), pilot studies were carried out testing the effects of mode of delivery of prostaglandin E1, E2, and F on subsequent intake of solutions bearing o-aminoacetophenone (OAP). OAP (Fig. 1) was chosen as the test irritant because it is an especially potent avian repellent. The preliminary studies suggested that prosta-

glandin E1 (PGE1) was most effective at sensitizing starlings to the effects of OAP, and that oral presentation of prostaglandin yielded the same effects as an IP injection. Oral delivery was preferred because it was less stressful to the birds.

Experiment 1: Effects of PGE1 on the dose-response of OAP

This experiment determined how orally administered PGE1 affected the dose-avoidance response for trigeminally mediated stimuli. The experimental design utilized a standard drinking assay (Clark and Shah, '91), and consisted of an adaptation/assignment, treatment, and test phase.

During the adaptation/assignment phase, birds were presented with tap water in calibrated Richter tubes on 5 consecutive days. Water intake was recorded every 2 hr for a total of 6 hr, after which the Richter tubes were replaced with standard water bottles. Water consumption for a 2-hr interval averaged 6.5 ml (\pm 0.37 SE). This rate of intake was within the normative range reported in previous studies (Clark and Shah, '91). Starlings ($n = 36$) were ranked on the basis of mean water consumption and assigned to six groups counterbalanced with respect to drinking. Analyses were segmented in an a priori fashion following von Eye ('90). Similarity for water consumption among groups was validated using a one-way analysis of variance ($P > .05$, one-way ANOVA), and was a prerequisite for further testing.

During the treatment phase, all starlings were presented with a 0.8- μ g/ml solution of PGE1, and intake was recorded after 2 hr. The concentration for PGE1 was based on anticipated fluid intake during a 2-hr period necessary to achieve PGE1 doses which yielded behavioral effects for rodents (Collier and Schneider, '72). Similarity of fluid intake among groups was verified ($P > .05$, one-way ANOVA).

Having met the criteria conditions, groups were assigned randomly to OAP concentration-groups (14, 7, 3.5, 0.7, 0.35, and 0.07 mM). Tests of the assumptions and replacement of the PGE1 drinking tubes with OAP-filled tubes were accomplished within 30 min. OAP intake was recorded after 2 hr. On the following days, starlings were observed for post-ingestional effects of the experiment (Clark and Shah, '91, '94). In the absence of obvious visual carryover effects (e.g., malaise, piloerection, lethargy), the experiment was repeated for the control treatment condition. The order of treatment (PGE1 or water control) was determined randomly. After the two tests, birds were

the group housing facility and were adapted, assigned to groups on the basis of water intake, and tested for similarity for water intake among groups as described in experiment 1. During the treatment phase, birds were presented with an aspirin solution (5 mM, see below) and consumption was recorded after 2 hr (Fig. 1). Again, similarity for group consumption was tested using a one-way ANOVA as a precondition for further testing and analysis.

During the test phase, groups were randomly assigned to receive one of six concentrations of OAP: 14.0, 7.0, 3.5, 0.7, 0.35, and 0.07 mM. Consumption of fluid was recorded after 2 hr, and standard water bottles were returned to the cages. Monitoring of post-test behavior for signs of carryover effects followed methods outlined for experiment 1. In the absence of any post-test effects, the experiment was repeated during the following days by substituting treatment level with a water control, and subsequently an 18.5 mM aspirin solution. Concentrations of OAP used for these tests were 28, 10, 1, 0.5, 0.1, and 0.05 mM. The order of treatment level was determined randomly. After the three experiments birds were returned to the group housing facility.

A comparison of the relative intake for the three treatment conditions ($n = 3$: 5 mM and 18.5 mM aspirin and the control) as a function of OAP concentration ($n = 6$) was made using a repeated-measures two-way ANOVA. The between-subjects effect was concentration-group, and the repeated measure was treatment. The dependent variable was the ratio of fluid intake of OAP divided by fluid intake during the 2 hr preceding OAP presentation.

Experiment 3: Sequence effects of irritant and drug

This experiment determined whether prior exposure to OAP might affect the ability of aspirin to alter a starling's subsequent responsiveness to OAP. Eighteen starlings were drawn from the group housing facility, adapted, ranked by water consumption, and assigned to one of three groups as described in experiment 1. Fluid intake was monitored at hourly intervals for 7 hr. The three treatment conditions consisted of the following sequences: (group 1) 3 hr of exposure to water, followed by 1 hr of exposure to a 5 mM solution of aspirin, followed by 3 hr of exposure to water; (group 2) 3 hr of exposure to 1.4 mM OAP, followed by 1 hr of exposure to a solution of 5 mM solution of aspirin, followed by 3 hr of exposure to 1.4 mM OAP; (group 3) 3 hr of exposure to 7

mM OAP, followed by 1 hr of exposure to 5 mM aspirin, followed by 3 hr of exposure to 7 mM OAP. Data were analyzed using a repeated-measures two-way ANOVA design, with hour as the repeated measure and treatment sequence as the between-subjects effect.

Experiment 4: Effects of aspirin on acetophenones

This experiment determined whether the analgesic effect of aspirin might extend to other acetophenone repellents. Three compounds were tested (Fig. 1). During the adaptation phase, 36 starlings were drawn from the group housing pool, ranked, and assigned to one of six groups ($n = 6$ /group) on the basis of water intake according to methods described in experiment 1. Mean water intake among groups was verified (one-way ANOVA).

During the treatment phase, three groups were assigned randomly to the control condition, i.e., they were presented with water during the treatment period. The remaining three groups were presented with a 5 mM solution of aspirin. Fluid intake was recorded after 2 hr, and similarity among the groups' fluid intake was verified (one-way ANOVA).

During the test phase, one group from within each treatment category (control or aspirin) was assigned randomly to receive one of three equimolar (7 mM) repellent solutions (AP, 2MOAP, OAP; Fig. 1). Intake was recorded after 2 hr. Data were analyzed using a two-way ANOVA. Drug ($n = 2$) and repellent ($n = 3$) were between-subjects factors. The dependent variable was repellent intake divided by the previous 2-hr fluid intake.

Experiment 5: Effects of various drugs on repellent intake

Other aspirin-like drugs were evaluated for their analgesic effects on starlings as a test for the generality of the effects on acetophenones. Six drugs were arbitrarily selected from a list of representative mammalian analgesics (Fig. 1; Ferreira and Vane, '74). Drugs were selected to represent a range of potencies and possible modes of action. Aspirin is a prostaglandin biosynthesis inhibitor (Vane, '71; Ferreira and Vane, '74; Kotwani et al., '94) that operates at the "local tissue level" at the site of irritation (Flower et al., '72). In contrast, acetaminophen and similar drugs have been shown to produce their analgesic effects centrally (Flower and Vane, '72).

During the adaptation phase, 18 starlings were selected from the group pool, ranked on the basis

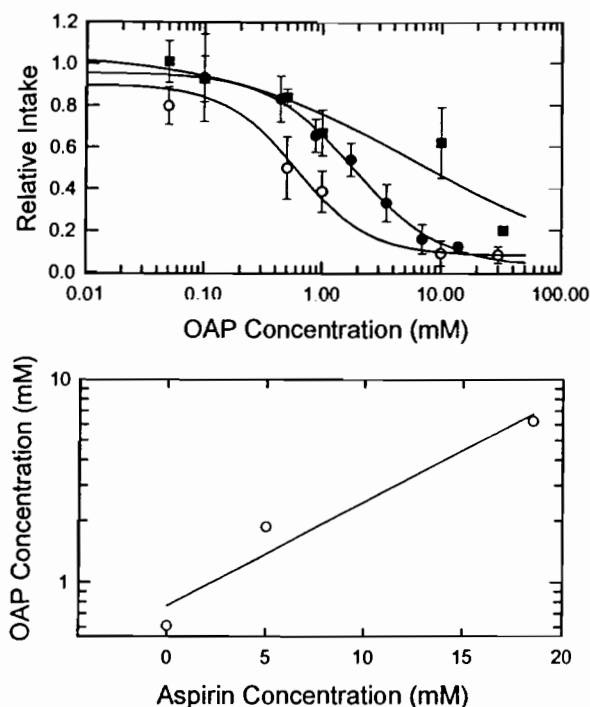


Fig. 3. **Top:** The OAP dose-response curves for starlings self-medicated with 0 mM (open circles), 5 mM (solid circles), and 18.5 mM (solid squares) aspirin solutions. **Bottom:** The relationship between the inflection of the OAP dose-response and aspirin dose. Vertical bars depict standard error estimates.

Experiment 3: Sequence effects of irritant and drug

Fluid intake profiles for the three treatment sequences differed (Fig. 4, two-way interaction, $F = 3.59$, $df = 12,90$, $P < .001$). Intake of tap water was within the range of fluid intake normally found for control groups. Furthermore, intake for the control condition did not differ between the period prior to aspirin presentation, during aspirin presentation, or after aspirin presentation ($P > .05$). Hourly mean fluid intake was $3.0 \text{ ml} \pm 0.5 \text{ SE}$, $3.3 \text{ ml} \pm 0.7 \text{ SE}$, and $3.22 \text{ ml} \pm 0.4 \text{ SE}$, respectively.

Fluid intake was suppressed by OAP as a function of its concentration (treatment main effect, $F = 6.02$, $df = 2,15$, $P = .0120$). There was also a clear time effect ($F = 11.80$, $df = 6,90$, $P < .001$). Post-hoc tests suggested the following pattern. OAP intake was not affected by aspirin presentation if the birds were already exposed to OAP. Moreover, aspirin had no aversive quality as evidenced by an increased fluid consumption during its availability, presumably as an effort to com-

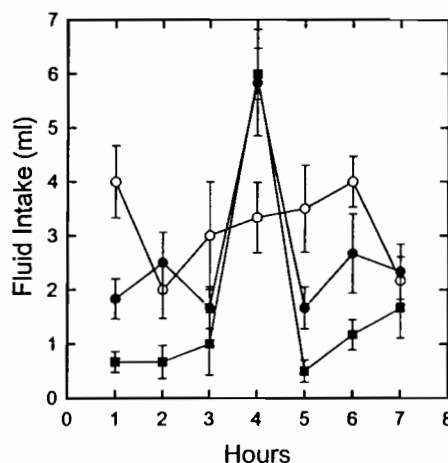


Fig. 4. The hourly fluid intake of starlings presented with the treatment sequence of repellent-aspirin-repellent (7 mM OAP, solid squares and 0.6 mM OAP, solid circles), or the control condition water-aspirin-water (open circles). Vertical bars depict standard error estimates.

pensate for moderate water deprivation resulting from exposure to OAP. Hourly mean intake for a 1.4 mM OAP solution prior to aspirin presentation was $2.0 \text{ ml} \pm 0.3 \text{ SE}$; during aspirin presentation intake was $5.8 \text{ ml} \pm 1.1 \text{ SE}$; and after presentation 1.4 mM OAP intake was $2.2 \text{ ml} \pm 0.3 \text{ SE}$. Similarly hourly mean intake for a 7.0 mM OAP solution prior to aspirin exposure was $0.8 \text{ ml} \pm 0.2 \text{ SE}$; during aspirin exposure it was $6.0 \text{ ml} \pm 0.5 \text{ SE}$; and after aspirin presentation it was $1.1 \text{ ml} \pm 0.3 \text{ SE}$.

Experiment 4: Effects of aspirin on acetophenones

Intake for a 5 mM aspirin solution was similar to that of the control group for each of the three experiments (AP, $P = .715$; OAP, $P = .150$; 2MOAP, $P = .388$). Having met the criterion condition for each experiment, i.e., no drug effect, the experiments proceeded to the test phase.

The desensitization effects of aspirin may extend to other acetophenone compounds (Fig. 5). Relative to controls, starlings medicated with aspirin increased consumption of the unsubstituted base molecule, acetophenone ($t = 2.35$, $df = 10$, $P = .041$) and, as seen in experiment 2, the amino substituted moiety, OAP ($t = 2.35$, $df = 10$, $P = .047$). Aspirin had no effect on responsiveness to 2MOAP ($P = .478$), although there was a tendency toward desensitization.

Lim, '62; Guzman et al., '64). Together these results are consistent with the interpretation that prostaglandins are involved in the modulation of the avian pain/irritation response, and that the physiological mechanism involving prostaglandins is similar to that described in mammalian models (Ferreira and Vane, '74; cf. Kotwani et al., '94; Chromiak and Vandenburg, '94).

Actual differences in the effectiveness of drugs to produce analgesia in starlings may be attributed to variation in rates of drug uptake and clearance (Erickson, '87). However, the tendency for benzoic acid to desensitize birds to the effects of OAP suggests that the carboxylic acid function of the benzoic acid moiety or aspirin-like drugs may be involved in the desensitization effect. This is consistent with mammalian models showing the relationship between analgesia and benzoic acid derivatives (Murthy et al., '82; Rafferty and Johnson, '87; Delaney, '90). Analgesic action for aspirin-like drugs is achieved through prostaglandin biosynthesis inhibition at the local tissue level. The apparent inability of acetaminophen and piroxicam to desensitize starlings to the effects of OAP suggests that modulation of the avoidance response is not centrally mediated because these drugs tend to act via central nervous system influence on pain perception (Flower and Vane, '72).

Because the process for modulating the perception of irritation is similar in birds and mammals, but the sensitivity to specific stimuli differs, the inference is that stimulus codes (i.e., stimulus-receptor mechanism) differ between the two taxa. Structure-activity studies suggest modest differences and some similarities between birds and mammals in receptor mechanisms for aromatic structures (cf. Nielsen, '91; Clark and Shah, '94). For example, it appears that birds and mammals share a benzene moiety binding site that is responsible for activating the sensory afferent (Szolcsanyi and Jancso-Gabor, '75; Nielsen, '91; Shah et al., '91; Clark and Shah, '94). In mammals, activation is facilitated, possibly owing to an associated thiol site that can bind to a long-chain alkyl group attached to the phenyl group. The alkyl structure helps the benzene maintain proper orientation for receptor activation (Nielsen, '91). However, birds do not respond to capsaicin (Szolcsanyi et al., '86; Mason et al., '91; Norman et al., '92). Removal of the alkyl substituent activates the stimulus as an avian irritant but inactivates the molecule as a mammalian irritant (Mason et al., '91). Proper orientation of the benzene moiety for avian irritants may be maintained

by the electrostatic charge distribution of the aromatic's substituents (Clark and Aronov, unpublished). Whether the prostaglandin effects of lowering threshold sensitivity toward OAP are mediated through a direct effect on a putative OAP receptor or an endogenous receptor mechanism (e.g., bradykinin receptor) are unknown and warrant further study. A better understanding of the mechanistic differences for sensory perception of birds and mammals will lead to a better appreciation of the constraints on feeding choices for the two taxa and lead to a better insight into the potential impact differences in sensory perception have on the evolution of plant-animal interactions.

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LITERATURE CITED

- Chromiak, J.A., and H.H. Vandenburg (1994) Mechanical stimulation of skeletal muscle cells mitigates glucocorticoid-induced decreases in prostaglandin production and prostaglandin synthase activity. *J. Cell. Physiol.*, 159:407-414.
- Clark, L., and P.S. Shah (1991) Nonlethal bird repellents: in search of a general model relating repellency and chemical structure. *J. Wildl. Manage.*, 55:538-545.
- Clark, L., and P. Shah (1994) Tests and refinements of a general structure-activity model for avian repellents. *J. Chem. Ecol.*, 20:321-339.
- Clark, L., P.S. Shah, and J.R. Mason (1991) Chemical repellency in birds: relationship between chemical structure and avoidance response. *J. Exp. Zool.*, 260:310-322.
- Collier, H.O.J., and C. Schneider (1972) Nociceptive response to prostaglandins and analgesic actions of aspirin and morphine. *Nature* 236:141.
- Delaney, N.G. (1990) Preparation of aminobenzoic acid and aminocyclohexanecarboxylic acids as neutral endopeptidase inhibitors and pharmaceutical compositions. European Patent Appl. EP 361,365.
- Dolbeer, R.A., M.A. Link, and P.P. Woronecki (1988) Naphthalene shows no repellency for starlings. *Wildl. Soc. Bull.*, 16:62-64.
- Erickson, H.H. (1987) Pain, animal. In: *Encyclopedia of Neuroscience*. G. Adelman, ed. Birkhauser, Boston, pp. 898-899.
- Ferreira, S.H. (1972) Prostaglandins, aspirin-like drugs and analgesia. *Nature* 240:200.
- Ferreira, S.H., and J.R. Vane (1974) Aspirin and Prostaglandins. In: *The Prostaglandins*. P.W. Ramwell, ed. Plenum Press, New York, pp. 1-48.
- Ferreira, S.H., S. Moncada, and J.R. Vane (1973) Prostaglandins and the mechanism of analgesia produced by aspirin-like drugs. *Br. J. Pharmacol.*, 49:86.